An *in vitro* study on the assessment of xanthine oxidase inhibitory potential of *Coriandrum sativum* extract

Type of study: Original research
Running title: Xanthine oxidase inhibitory activity of *Coriandrum sativum*

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ABSTRACT :

Introduction:

Coriandrum sativum is a member of the Apiaceae family, is among most widely used medicinal plants, possessing nutritional as well as medicinal properties.*Coriandrum sativum* is a summer annual plant commonly used as fresh green herb, spice, or for its essential oil.Gout is a disease associated with deposits of uric acid in kidney It can be extremely painful and incapacitating but is extremely treatable in almost all patients. The xanthine oxidase inhibitors are used to decrease the serum urate levels in patients with gout.The goal of this research is to see if *Coriander sativum* extract has anti-gout properties.

Materials and methods: Ethanolic extract was prepared. The phytochemical screening antioxidant and xanthine oxidase inhibitory activity was studied using the plant extract. The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Duncans's multiple range test, to see the statistical significance among the groups. The results with the $p<0.05$ level were considered statistically significant.

Results: Phytochemical screening showed that the extract is rich in carbohydrates, steroids, saponins, terpenoids, alkaloids, flavonoid and proteins. DPPH radical scavenging activity showed the potent in vitro antioxidant activity of the plant extract. The extract also possessed a dose dependent increase in the xanthine oxidase inhibitory potential. Xanthine oxidase inhibitory of the extract might have revealed the antigout activity of plant.

Conclusion: The study reveals that antigout and antioxidant activity of *Coriandrum sativum*. The presence of phytochemicals in the extract might be the underlying reason for the beneficial activity of the plant.

Keywords: Xanthine oxidase, *Coriandrum sativum*, antioxidant, phytochemical, gout,innovative technology; novel method.

INTRODUCTION :

Hyperuricemia characterised by high level of uric acid in blood due to increased activity of xanthine oxidase. Polyphenol compounds have been recognised to have many beneficial properties (1). Gout is associated with increased production and accumulation of uric acid crystals which is mainly associated with the increased activity of xanthine oxidase. Xanthine oxidase involved in metabolism of purine The final step of purine catabolism, the production of uric acid is catalysed by xanthine oxidase (2). Xanthine oxidase can also form free radical compounds such as superoxide anion and hydrogen peroxide. Xanthine oxidase plays a crucial role in the degradation of purine (3). Xanthine oxidase inhibitors play a crucial role in the treatment of gout and hyperuricemia. Allopurinol and has been clinically approved as an xanthine oxidase inhibitor to treat hyperuricemia and gout. However many undesirable effects such as hypersensitivity syndrome, hepatitis nephropathy, eosinophilia, vasculitis, fever, and skin rash are associated with the use of allopurinol (4,5).

Coriander is native to the Mediterranean which is used as a spice. The spices are well known for its effect on carbohydrate and lipid metabolism (6) . Coriander (*Coriandrum sativum L.*), a herbal plant of Apiaceae family, is valued for its culinary and medicinal uses. This plant plays an important role in the usage of flavouring agents and many different disorders in the folk medicine system are treated in different civilizations. Methanolic extract of coriander reported to have antibacterial activity.

Bioactive constituents of Coriander were found to be used in combination with conventional drugs. Potential antifungal activity of coriandrum sativum is also documented. Coriander as an herb is generally used in folk medicine as a cholesterol lowering agent, digestive stimulant and antihypertensive agent (7,8). Coriander extract is also proven to possess a strong antifungal effect against candida species(10) .

Gout is a metabolic disease that affects uricotelic animals that lack the uricase enzyme(10). As sodium urate crystals are deposited in joints, gout is usually associated with an elevated buildup of uric acid (11). Colchicine and Allopurinol are two gout medications that have been linked to a number of adverse side effects (9,12). Coriandrum sativum has not been examined for its in vitro xanthine oxidase inhibitory capability. (9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28)

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The aim of this research is to see if *Coriandrum sativum* extract has anti-gout properties by inhibiting Xanthine oxidase.

MATERIAL AND METHODS :

1. Phytochemical Screening test

1.1 Test for phlobatannin:

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

1. 2. Test for Carbohydrates :

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of red or dull violet rings at the junction of the liquids showed the presence of carbohydrates.

1. 3. Test for Flavonoids :

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

1.4. Test for Alkaloids :

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

1. 5. Test for Terpenoids:

2 ml of sample along with 2ml of chloroform and 3ml of con. H2SO4 was added. Red color ppt obtained indicates the presence of terpenoids.

1. 6. Test for proteins :

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins :

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

1.7. Test for steroids :

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2. DPPH free radical scavenging activity of Coriandrum sativum

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

DPPH radical scavenging (%) = Control OD-Sample OD X100 / Control OD

3. *In vitro* Xanthine Oxidase Inhibitory Activity of Coriandrum sativum

In vitro Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007). Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25◦C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25◦C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25◦C. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

Inhibitory activity (%) = (1 - As/Ac) x100 Where,

As – absorbance in presence of test substance, Ac – absorbance of control

4. STATISTICAL ANALYSIS :

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

RESULTS AND DISCUSSION:

Table 1 : Phytochemical analysis of *Coriandrum sativum* extract

Carbohydrates	++

Terpenoids	++
Amino acid	+
Flavonoids	+
Alkaloids	+
Steroids	++
Saponins	++
Protein	+

The qualitative phytochemical analysis of *Coriandrum sativum* leaf extract was done. The results showed that the extract is rich in carbohydrates, terpenoids, steroids, and saponins, and is moderately rich in flavonoids, alkaloids, and proteins.

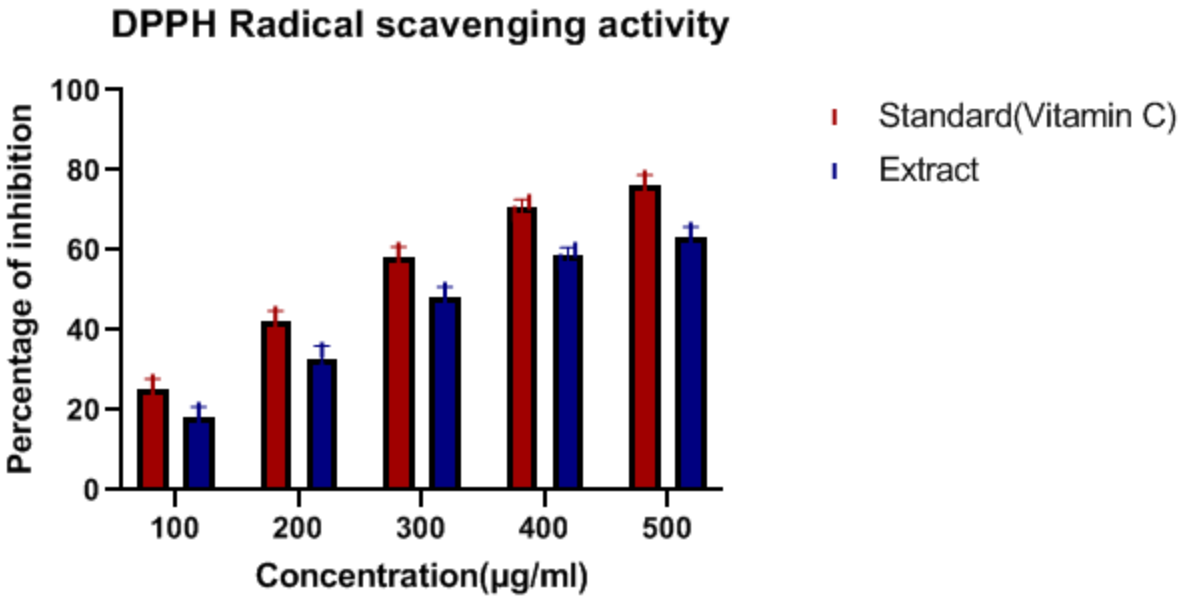

Figure 1: This graph represents the in vitro antioxidant activity of Coriandrum sativum as compared to its standard (Vitamin C). Each line represents the mean± SEM of 3 independent observations. X axis represents concentration and the Y axis represents the percentage of inhibition. Green colour denotes Coriandrum sativum and blue colour denotes standard (vitamin C). $p<0.05$ is considered to be statistically significant.

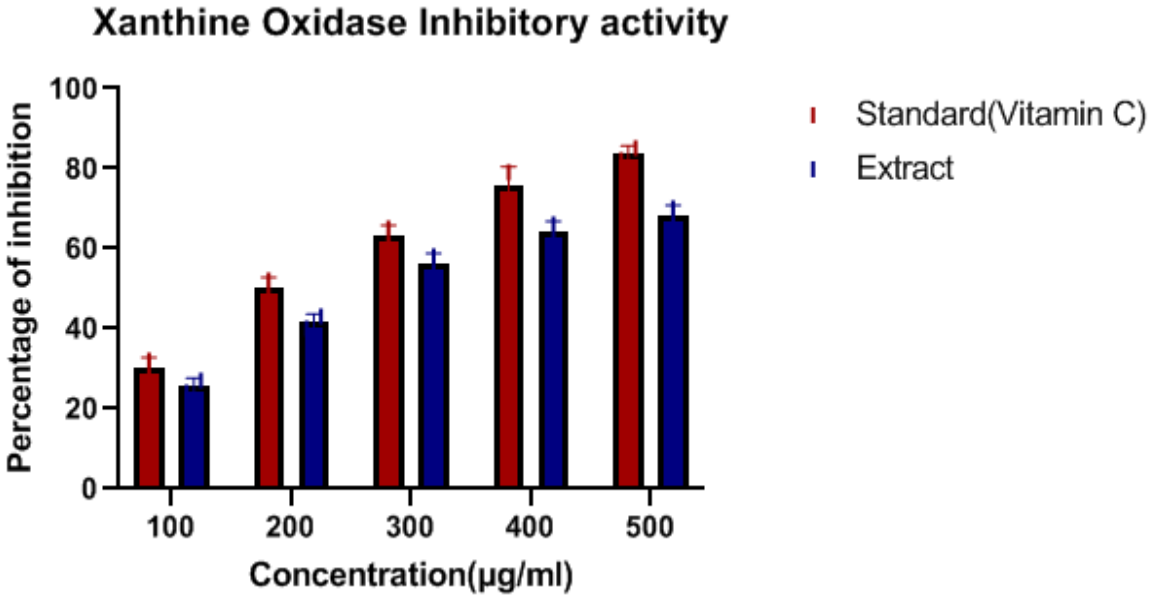

Figure 2: The graph represents the in vitro xanthine oxidase inhibitory activity of Coriandrum sativum as compared to the standard (Allopurinol). Each line represents the mean ± SSEM of 3 independent observations. The-axis represents concentration and the Y axis represents the percentage of inhibition. Orange colour denotes Coriandrum sativum and blue colour denotes standard (Allopurinol). P value <0.05 is considered to be statistically significant.

The phytochemical analysis of the extract showed that the extract is rich in carbohydrates, terpenoids, steroids, and saponins, and is moderately rich in flavonoids, alkaloids, and proteins (Table 1). The in vitro antioxidant activity of *Coriandrum sativum* extract was evaluated by DPPH radical scavenging activity. The results showed that the extract showed a dose-dependent increase in the radical scavenging activity, which indicates the antioxidant activity of the extract. But the activity is less as compared to that of the standard vitamin C (Figure1). The assessment of in vitro xanthine oxidase inhibitory potential of the plant extract revealed that the extract is capable of inhibiting the xanthine oxidase activity, and the activity increases with increase in concentration. Here also the activity of the extract is lesser than the standard drug allopurinol (Figure 2).

Phytochemicalsin the plants are having a great deal of attraction. This is mainly due to its role in preventing deadly diseases caused as a result of oxidative stress, and release of reactive oxygen species (29). The results of our study also revealed the presence of many phytochemicals which might have contributed to the pharmacological activities of the extract. Previous study also showed that the Coriander sativum extract was found to be rich in phytochemicals such as phenols, flavonoids and Alkaloids (1). Antioxidants are compounds that can prevent and stabilize the damage caused by free radicals by donating electrons from antioxidants to these damaged cells. Antioxidants also help to also conert free radicals into waste by-products, which can be eliminated from the body. Consumption of antioxidant-enriched fruits and vegetables is known to lower the risk of several diseases caused by free radicals (30). Plant extracts which are rich in phenolic compounds exhibit a good antioxidant potential. From the previous studies, it is evident that the aromatic rings possessed by the phenolic compounds help in the elimination of free radicals and thus the plant extracts rich in alkaloids and flavonoids can be good representatives to exhibit antioxidant potential (31,32). Our extract also showed a good antioxidant capacity which might be due to the presence of the phytochemicals present in the extract.

Anti Gout potential is the herb's ability to inhibit the enzyme xanthine oxidase. Xanthine oxidase acts as a key enzyme in the production of uric acid. Inhibition of xanthine oxidase inhibits the production of uric acid and helps to relieve the clinical condition called Gout (33). In this study, Coriander extract was found to possess xanthine oxidase inhibitory potential in all the tested concentrations, which indicate its antigout activity. Allopurinol is a xanthine oxidase inhibitor which is clinically used for the treatment of gout. (34). However, usage of this drug is associated with many side effects such as hepatitis, nephropathy, and allergic reactions (35)..This study forms only a platform for the antigout activity of the extract. Only in vitro analysis was done. Detailed in vivo studies can be done to

understand the exact mechanism of action of the extract. The isolation of active principle from the plant can help to develop a therapeutic drug from the plant. Thus, the search for novel XO inhibitors with a greater therapeutic activity and lesser side effects have to be developed not only to treat gout but also to combat various other diseases associated with the XO activity. Thus detailed studies in our plant will be a way to understand the exact mechanism of antigout activity and to develop a drug.

CONCLUSION :

The ethanolic extract of *Coriandrum sativum* has significant antioxidant and xanthine oxidase inhibitory activity, according to this study. The presence of phytochemicals in the extract could be the reason for the plant's therapeutic properties.

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STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study

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